The Agricultural Health Study Biomarker Workshop on Cancer Etiology

Introduction: Overview of Study Design, Results, and Goals of the Workshop

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Received 1 April 2005; accepted 6 April 2005

The Agricultural Health Study is a long-term prospective cohort study of 89,658 subjects (58,564 in Iowa and 31,094 in North Carolina), approximately 57,000 private and commercial pesticide applicators and 32,000 spouses of private applicators. The central focus of this study is to identify the exposures in the agricultural environment that are responsible for the excess cancers and other chronic diseases that have been observed in this cohort and other agricultural populations throughout the world. Because of its large size, prospective design, detailed and repeated exposure assessments, the Agricultural Health Study is a unique resource with which we can investigate these important public health questions.

Enrollment into the study entailed completion of a long self-administered questionnaire concerning specific pesticide use, work practices, occupational histories, medical history, and lifestyle characteristics. At the time of enrollment all subjects were free of cancer, avoiding case recall bias. Enrollment began in December 1993, and was completed in December 1997 (phase I). A second round of questionnaires was administered by computer-assisted telephone interview between 1998 and 2003. This effort updated pesticide exposure histories and medical histories. During this phase of the study (phase II), we also collected buccal cell samples from approximately 33,000 study subjects and sputum samples from approximately 22,000 of these same study subjects. A third round of interviews is scheduled to begin in November 2005.

Enrollment and continued participation in the study has been very high, with less than 1% of the study populations being lost to follow-up. To date we have accumulated 620,000 person-years of observation, and during this period we have observed over 3,700 incident cancer cases. While the overall cancer incidence is significantly lower than the cancer incidence in Iowa and North Carolina overall, significant excesses of leukemia, non-Hodgkins lymphoma, and cancers of the prostate, lung, colon, and rectum have been observed among those using a number of different pesticides.

The first paper to explore pesticide exposure and a cancer outcome was published in early 2003 and it focused on the incidence of prostate cancer [1]. Of the 50 pesticides examined, only methyl bromide was shown to have a statistical association with prostate cancer, with increasing exposure (p trend = 0.0004). In the highest exposure category, the OR was 3.47 (95% CI = 1.37–8.76) compared with the nonexposed. This association was consistent between Iowa and North Carolina. Although the US EPA classifies methyl bromide as not likely to be a human carcinogen, NIOSH considers it a potential occupational carcinogen. In addition, several pesticides, including coumaphos, 2, 2-dichloroethenyl dimethylphosphate, fonofos, permethrin (animal use), and phorate were associated with prostate cancer among those applicators who reported a family history of prostate cancer.

Since that time, seven other cancer etiology papers have been published, including two other disease-specific analyses examining cancer of the female breast and lung. None of the 50 pesticides examined was

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clearly associated with the breast cancer risk among farmers' wives [2]. Seven pesticides (dicamba, metolachlor, pendimethalin, carbofuran, chlorpyrifos, diazinon, and dieldrin), however, were associated with lung cancer risk in the AHS [3]. For dicamba, chlorpyrifos, and carbofuran, the association was most evident when the low-exposed were used as the referents. For the remaining four pesticides (metolachlor, pendimethalin, diazinon, and dieldrin), the associations were not monotonic and were most evident in the highest exposure categories, although the tests for linear trend were significant.

Two of the seven pesticides (chlorpyrifos and carbofuran) that were associated with lung cancer have been the subject of published pesticide-specific analyses where other tumor sites were examined in addition to lung cancer. Of the other tumor sites examined (rectal, brain, esophagus, kidney, NHL, leukemia, multiple myeloma, and all lymphohematopoietic cancer combined), none was associated with occupational use of chlorpyrifos [4]. For lung cancer, however, the association with chlorpyrifos was relatively consistent between Iowa and North Carolina. However, the association appeared to be limited to current smokers only. The US EPA classifies chlorpyrifos as having evidence of noncarcinogenicity for humans.

Carbofuran was only associated with lung cancer when the low-exposed group was used as the referent [5]. The association was largely consistent between Iowa and North Carolina and was limited to former and current smokers (only one lung cancer case occurred among never smokers). The US EPA classifies carbofuran as not likely to be carcinogenic to humans.

The pesticide specific analysis of alachlor suggested an exposure-response trend for lymphohematopoietic cancers combined, but not with any other tumor site examined [6]. Alachlor has been shown to induce malignant tumors in rats [7–9] and the US EPA classifies alachlor as a probable human carcinogen at high doses.

After an average of 6 years of follow-up, there was no indication that atrazine [10] or glyphosate [11] were associated with any of the tumor sites examined. Although no associations were observed for these pesticides, we would be unable to identify modest increases in risk because of the relatively small number of exposed cases of many tumor sites examined. In addition to these eight publications, we expect to publish approximately 10 additional papers of this type every year for the next 4 years.

It seems clear, therefore, that the Agricultural Health Study is beginning to have a major influence on the scientific evaluation of pesticide safety and this strong influence will continue for years to come. This situation accentuates our responsibility to do all we can to minimize both the error of falsely identifying a pesticide as a human carcinogen when it is not, as well as failing to identify a pesticide as a carcinogen when it is, in fact, a carcinogen.

Within the design of the Agricultural Health Study we have several safeguards in place to help us avoid making either a false positive or a false negative association between exposure to a specific pesticide and cancer:

First, we look to see if we have a consistency across geographical area, namely, is there a dose-response effect in both Iowa and North Carolina,

Second, we look to see if we find the same dose-response in private applicators and commercial applicators,

Third, after an initial significant positive association, we look to see if we can duplicate the finding at a second period of time. For example, the association we found between certain pesticides and prostate cancer was observed in 566 incidents of prostate cancer cases diagnosed between 1993 and 1998. Now that we have approximately 500 new incident cases diagnosed between 1999 and 2002 and we are in the process of reevaluating the association. We will conduct similar analyses for other organ sites as sufficient cases accumulate.

Additionally, we have been collaborating with the USEPA and NIOSH to validate our questionnaire-based estimates of exposure to specific pesticides by conducting field measurements of both external and internal exposure. Preliminary results indicate that our questionnaire-based estimates of pesticide exposure accurately rank order of both external and internal pesticide exposures among the cohort members. If these evaluations of our questionnaires continue to demonstrate the validity of our rank-order exposure classifications, cancer risk estimates resulting from our work will have minimized the possibility of either false positive or false negative associations.

Evaluating the biological plausibility of our epidemiological associations linking particular pesticides with particular cancers in well-designed biomarker studies is our final safeguard against false positive or false negative associations. It is for that purpose that this workshop was organized. The human toxicological literature concerning pesticides has grown considerably in the past few years and the Agricultural Health Study is now in a position to incorporate the best ideas into nested biomarker studies that would directly assess human toxicity and the plausibility of our epidemiological associations.

Whenever possible, we would like to encourage collaborations to further evaluate our study findings linking specific pesticides to specific cancers. Studies evaluating the link between exposures and early biological effect, chronic biological effect, or to preclinical

markers of disease would add valuable detail to our proposed causal association. Whenever possible we would also like to support studies that measure cancer susceptibility.

In some of the epidemiological literature these biomarker studies are called "transitional studies" and they occur in three broad types. "Developmental studies" are studies that attempt to develop a biomarker for use in human populations. These studies are frequently an outgrowth of experimental studies in animals. These developmental studies evaluate biological sample collection, processing, and storage procedures. They also evaluate the accuracy and precision of the assays. "Characterization studies" are usually the next step among transitional studies and their purpose is to assess the range of the biomarker in representative populations. Finally, there are the "applied studies" that evaluate the exposure–biomarker relationship or the exposure–biomarker of susceptible relationships. Although all these studies are necessary to achieve the end result, the Agricultural Health Study will be most interested in supporting the "applied studies" because our focus has been and must continue to be the evaluation of cancer etiology.

This brings us to the goals of the biomarker workshop that are to

1. Evaluate the potential for mechanistic toxicology to contribute to the Agricultural Health Study goal of identifying carcinogenic pesticides.
2. Evaluate the appropriateness, feasibility, and timeliness of conducting molecular epidemiology studies in the AHS.
3. Prioritize the hypotheses that should be investigated in such studies.
4. If appropriate (considering items 1–3), proposing specific add-on studies within the AHS to investigate pesticide-related carcinogenic mechanisms.
5. Explore intramural and extramural mechanisms by which this research can be conducted.
6. Publish a state-of-the-art review paper evaluating the current state of knowledge from epidemiological and toxicological studies of pesticides and cancer, based on the proceedings of this workshop.

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